JSR Publications

Available Online

J. Sci. Res. **4** (1), 203-212 (2012)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Antimicrobial Activity of a Halophytic Plant *Cressa cretica* L.

P. Sunita[1*], S. Jha[2], S. P. Pattanayak[3], and S. K. Mishra[4]

[1]Govt. Pharmacy Institute, Department of Health Education and Family Welfare, Govt. of Jharkhand, Ranchi, India

[2]Division of Pharmacognosy, Birla Institute of Technology, Mesra, Ranchi-835 215, Jharkhand, India

[3]Division of Pharmacology, Birla Institute of Technology (BIT), Ranchi-835 215, Jharkhand, India

[4]University Department of Pharmaceutical Sciences, Utkal University, Vanivihar, Odisha, India

Received 24 May 2011, accepted in revised form 17 October 2011

Abstract

In the present investigation different fractions (hexane, ethylacetate and methanol) of the whole methanolic extract of *Cressa cretica* L.(Convolulaceae), a widely grown halophytic plant were studied against wide ranges of bacteria (both positive and negative strain) and five fungi *Candida albicans, Candida tropicalis* – dimorphic fungi*, Aspergillus fumigatus, Aspergillus niger* – systemic fungi and fusarium oxysporum by agar disc diffusion method. Among the three fractions the ethylacetate fraction of *C.cretica* showed the highest activity, but among the pathogens highest activity was revealed against *Escherichia coli, Klebsiella pneumoniae* (zone of inhibition diameter in mm was found to be 26 and 31, respectively). The ethylacetate fraction was sensitive to both gram +ve and gram –ve bacterias. *C.cretica* showed higher inhibitory activity against the *Aspergillus fumigates***,** *Aspergillus niger* (zone of inhibition diameter in mm was found to be 26 and 22, respectively) than the *Candida albicans* and *Candida tropicalis* and least was found for *Fusarium oxysporum*. As ethylacetate fraction revealed better activity than other fractions, further study of morphological and biochemical alterations were carried out only with ethylacetate fraction and the results revealed some morphological and biochemical alterations, which indicates that ethylacetate fraction of *C. cretica* possess potential broad spectrum antimicrobial activity.

Keywords: Cressa cretica; Antifungal; Gram positive bacteria; Gram negative bacteria; Biochemical alterations.

© 2012 JSR Publications. ISSN: 2070-0237 (Print); 2070-0245 (Online). All rights reserved.

doi:10.3329/jsr.v4i1.7719 J. Sci. Res. **4** (1), 203-212 (2012)

1. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs.

[*] *Corresponding author*: priyashree_lenka@yahoo.com

Therefore, such plants should be investigated to better understand their properties, safety and efficacy [1]. Infectious diseases are the leading cause of death worldwide. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens [2]. Increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have lead to screening of several medical plants for their potential antimicrobial activities. There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared form plants [3]. In modern days the antimicrobial activities of plant extract have formed the basis of many applications in pharmaceuticals, alternative medicines and natural therapy influence the health. Some of the active principles of bioactive compounds are preferred for their therapeutic purposes either singly or in combination to inhibit the life processes of microbes [4, 5]. With increase in antibiotic resistance, cost and inaccessibility (especially in rural areas) to some orthodox modern antibiotics, traditional weeds are fast gaining popularity even to urban and civilized dwellers.

Cressa cretica L. (Convolulaceae), popularly known as 'Rudanti' in Hindi and is a widely grown halophytic plant. Different parts of the plant have been claimed to be valuable in a wide spectrum of diseases [6-8]. In earlier studies *C. cretica* Linn flowers exhibited cytotoxic and anti-inflammatory activity in vitro [9]. *C. cretica* is reported to be antibilous, antituberculosis, and expectorant [10]. Shahat *et al.* [11] yielded five flavonoids (quercetin, quercetin-3-O-glucoside, kaempferol- 3-O-rhamnoglucoside, and rutin) from the aerial parts of *C. cretica*. It is also reported the fruits of *C. cretica* is a potential source of edible oil. The oil of *C.cretica* was free from any undesirable components and could safely be recommended for human consumption. Recently we have reported the anti-inflammatory activity of the plant and it's *in-vivo* and *in-vitro* antioxidant activity. The plant also possesses anti-tussive activity in experimental rats [12-14]. The plant is used as stomachic, tonic and aphrodisiac purposes, enriches the blood, and is useful in constipation, leprosy, asthma, and urinary discharges and anthelmintic, in the treatment of diabetes and general debility [15].

An attempt has been made to study the antimicrobial activity against of different fractions of *C. cretica* against microorganisms: *Staphylococcus aureus, Staphylococcus pyogenes, Streptococcus pneumonia, Micrococcus luteus, Bacillus subtilis, Bacillus cereus*, *Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium*, *Salmonella typhi, Pseudomonas aeroginosa,* and *Serratia marcescens* and fungus: *Candida albicans, Candida tropicalis, Aspergillus fumigates, Aspergillus niger, Fusarium oxysporum.* In addition, the morphgological and biochemical alterations were also carried out to the fraction which revealed maximum activity.

2. Materials and Methods

2.1. *Plant material*

Cressa cretica was collected from Nalban island of Chilika lake, Orissa, India and was preliminarily identified at Department of Natural Product, Institute of Mineral and Material Technology, (formerly known as Regional Research laboratory, Bhubaneswar) India, which was later on confirmed from Botanical Survey of India, Howrah, West

Bengal, India (CNH/I-I/32/2010/Tech.II/237). A voucher specimen has been kept in our laboratory for future reference.

2.2. ***Preparation of extract***

The plant parts were air-dried, pulverized to a coarse powder in a mechanical grinder, passed through a 40-mesh sieve and extracted in a soxhlet extractor with methanol. The extract was decanted, filtered with Whatman No. 1 filter paper and concentrated at reduced pressure below 40◦C through rota-vapor to obtain dry extract (16.73%w/w). *Cressa cretica* methanolic extract (CME) was adsorbed on to the 250 g of silica gel of 60–120 mesh size and fractionated using solvents of increasing polarity such as hexane (Fr-He), ethylacetate (Fr-Et), and methanol (Fr-Me). The fractions were subjected for preliminary phytochemical screening to show the presence of steroid, alkaloid, glycoside, tannin, triterpenoid, carbohydrates reducing sugar, and fatty acids.

2.3. ***Microorganisms***

The test microorganisms used for the antimicrobial activity were *Staphylococcus aureus* (MTCC 740), *Staphylococcus pyogenes* (MTCC 1924), *Staphylococcus epidermidis* (MTCC 2639), *Micrococcus luteus* (MTCC 2452), *Bacillus subtilis* (MTCC 619), *Bacillus cereus* (MTCC 430), *Escherichia coli (*MTCC 52), *Klebsiella pneumoniae*(MTCC 7407), *Salmonella typhimurium* (MTCC 98), *Salmonella typhi* (MTCC 3216), *Pseudomonas aeroginosa* (MTCC 424), *Serratia marcescens* (MTCC 3124), *Candida* albicans (MTCC 227), *Candida tropicalis* (MTCC 230), *Aspergillus fumigatus* (MTCC 343), *Aspergillus niger* (MTCC 478), *Fusarium oxysporum* (MTCC 2087)

2.4. ***Drugs and microbiological media***

The antimicrobial agents used were: amoxicillin (Smithkline Beecham Pharmaceuticals, U.K). ciprofloxacin (Ranbaxy Pharmaceuticals India) gentamycin(Ranbaxy Pharmaceuticals India), tetracycline(Microlabs India); ketoconazole (Dr. Reddy's Lab Ltd India); fluconazole (Greenlife pharmaceuticals India; nutrient broth and nutrient Agar (Oxiod Ltd, Basingtone, Hampshire, England) and Sabouraud Dextrose Agar (4% GlucoseAgar – Fluka Chemie GmBH CH-9471 Backs)

2.5. ***Antimicrobial activity & minimum inhibitory concentration (MIC)***

The antibacterial assay for screening of different fractions of the plant *C.cretica* were evaluated by the method of agar disc diffusion method [16]. The media (Muller Hinton Agar No-2 and MRS media and Sabroued dextrose agar for fungal strain) and the test bacterial cultures were poured into petridishes (Hi-media). The test strain (200µl) was inoculated into the media (inoculums size 10^8 cells/ml) when the temperature reached 40-42^0C. The test compound 100 µl was impregnated into sterile disc (7mm) (Hi-media) and was then allowed to dry. The disc was then introduced into medium with the microbes.

The plates were incubated overnight at 37^0C. Microbial growth was determined by measuring the diameter of the zone of inhibition. The experiment was performed in triplicate and the mean values of the result are shown in Table 1.

The disc (6mm in diameter) was impregnated with 10 µl of 100 mg/ml (1 mg/disc) *C. cretica* fractions placed on seeded agar. Gentamicin (10µg/disc), and tetracycline (10µg/disc) were used as positive controls for bacteria and fluconazole (10 µg/disc), ketoconazole (10 µg/disc) for fungi. The test plates were incubated at 37 ^{0}C for 24 h for bacteria and at 28^0C for 72 h for fungi depending on the incubation time required for a visible growth. Minimum inhibitory concentration (MIC) values were also studied for microorganisms, which were determined as sensitive to the fractions of *C.cretica* in disc diffusion assay. Sterile filter paper discs (6mm in diameter) containing 2.5–1000 µg/disc of all the components were placed on the surface of a medium. MIC was defined as the lowest concentration of extract that inhibited visible growth on agar.

2.5. *Morphological and biochemical alterations*

To study the morphological alterations, a single colony of each type of culture viz. *S. aureus, S. typhi, S. typhimurium, and E. coli* was inoculated in 5ml of nutrient broth (0.5% peptone, 0.3% beef extract, and 0.5% NaCl) and incubated at 37°C for 18-24h. This primary culture was used to inoculate the secondary broth cultures with $1x10^9$ cells/ml and the respective inhibitory concentration of *C.cretica* used was used for evaluation of biochemical tests**.** The control did not receive the plant extract. These secondary cultures were incubated at 37°C for 18h-24h. Gram staining and Hanging drop methods [17] were performed with secondary broth cultures and observed under oil immersion objective. To study the alterations in the colony morphology, secondary cultures of both control and experimental were placed on nutrient agar plates using serial dilution method and incubated at 37°C for 18-24h and observed. Briefly describing, the gram staining was done by preparing the smear of culture on a glass slide, and flooding with crystal violet, Gram's iodine, ethyl alcohol, and safranine for 30-45 sec. smear was washed with water after each of these steps. Later it was blotted, and oil drop was placed and observed under oil immersion objective. For hanging drop method a depression slide was used and a hanging drop of each culture was made from the cover slip and observed under oil immersion objective. Fermentation reactions were carried out to study acid and gas formation. The peptone water was inoculated with $1x10^9$ cells ml^{-1} of each culture type to which specific (10%) sugars such as lactose, glucose, dextrose, sucrose and mannitol (High Media, Mumbai, India) were added. To test the gas formation a Durham's tube was inserted in each test tube. These cultures were cultured in the presence (respective inhibitory concentrations) or absence of *C.cretica*, at 37°C for 24-48h. Later the acid formation was tested by addition of few drops of neutral red indicator to the cultures and formation of dark red color is scored as positive. Indole, methyle red and voges proskeur tests were perfomed by secondary cultures cultured in the presence or absence of *C.cretica* in peptone water and MRVP broth (Hi Media) respectively, and incubated at 37°C for 24-48h. Later Kovac's reagent was added to peptone water cultures and formation of cherry red colour was scored as positive. Similarly, methyl red and voges

proskeur reagents were added separately to MRVP broth cultures and formation of red color was scored as positive for methyl red and voges proskeur reactions respectively. Citrate utilization was tested by using Simmon's citrate agar slants that were streaked with the primary cultures in the presence and absence of extract and incubated at 37°C for 24h. The change of medium's green colour to blue is scored as positive. For all these tests, media controls with garlic but without addition of cultures were kept. To study the catalase activity overnight secondary cultures of both control and experimental were treated with 3% hydrogen peroxide taken in a capillary tube and extensive bubble formation in the tube was scored as positive. The oxidase activity was studied by placing the concentrated pellet of secondary broth control and experimental cultures on a piece of filter paper after which one or two drops of 1.0 % Wurster's reagent was placed. If the test culture turns purple it is scored as positive. Urease test was performed by culturing the secondary cultures of these pathogens in the presence and absence of *C.cretica* at 37°C for 18-24h in Christensen's urea broth (Hi Media). Later a few drops of Phenol red indicator was added to the cultures, and development of purple colour is scored as positive. Similarly the Deoxy ribonuclease activity was studied by streaking the Tolluidine blue Dnase agar plates (Hi Media) with a loop full of secondary broth cultures prepared in the presence and absence of *C. cretica*. These plates were incubated at 37°C for 18-24h. Formation of white colonies with light pink border and degradation of medium was scored as positive. The amylase, proteolytic and phospholipase-C activities were studied by line inoculating each type of bacteria on starch agar, milk agar, and egg yolk agar plates respectively in the presence and absence of *C.cretica* and incubating the plates at 37°C for 24 h. Then starch agar plates were flooded with iodine solution and white zone of hydrolysis around inoculum followed by blue non degraded part was scored as positive. The zone of degradation around inoculum on milk and egg yolk agar was scored as positive.

3. Result and Discussion

The results of preliminary phytochemical analysis of the plant indicated the presence of fixed oil, flavonoids, steroids, terpenes, and coumarins. Flavonoids are naturally occurring phenols which possess numerous biological activities including anti-microbial, anti-inflammatory, antiallegic, antithrombotic and vasoprotective effects [18, 19]. Coumarins are considered phytoalexins since they are produced by the plant as a defence mechanism against attack by other organisms [20,21]. Free 6-OH in the coumarin nucleus has been found to be important for antifungal activity, while the free hydroxyl group at position 7 is important for antibacterial activity [22]. Interestingly, coumarins have also inhibitory effect on DNA gyrase which may be linked to the anti- HIV (human immunodeficiency virus) activity [23]. The combination of these phyto-constituents may effectively inhibit the microorganisms.

3.1. *Antimicrobial activity and MIC*

Gram negative bacteria like *E.coli, P.aeruoginosa, S.typhi* may cause the urinary tract infections, GIT infections, skin infections etc. The gram positive organisms like *S. aureus,*

B.subtilis may cause the infections such as nosocomial infections, food poisoning, pyrarthirtis, endocardilis, suppurations, abscess formation, osteomyetilis and toxic shock syndrome etc. [24-27]. *Aspergillus niger* is food borne pathogens if inhaled with large amounts of spores, causes a serious lung disease, aspergillosis, otomycosis pain [28]. *C. albicans* which resides as commensal in the mucocutaneous cavities of skin, vagina and intestine of humans [29], can cause infections under altered physiological and pathological conditions such as infancy, pregnancy, diabetes, prolonged broad spectrum antibiotic administration, steroidal chemotherapy as well as AIDS [30-33].

Table 1. Antimicrobial activity of fractions on different bacterial and fungal strains by disc diffusion assay.

Sl. No	Microorganisms	Standard Antibiotics				Zone of Inhibition in diameter (mm)		
		Bactericide		Fungicide		Fraction A Fr-He	Fraction B Fr-Et	Fraction C Fr-Me
		Tetracycline (10µg/disc)	Gentamycin (10µg/disc)	Fluconazole (30µg/disc)	Ketoconazole (10µg/disc)			
				Bacterial strains				
1	*Staphylococcus aureus*	27.33±0.02	29.33±0.07	-	-	7.333±0.04	21.67±0.06	15.67±0.07
2	*Staphylococcus pyogenes*	30±0.03	28.67±0.12	-	-	6.667±0.06	21.33±0.16	14.33±0.72
3	*Staphylococcus epidermidis*	28.67±0.09	31.67±0.06	-	-	5.334±0.34	22.33±0.07	16.33±0.04
4	*Micrococcus luteus*	31±0.07	32±0.09	-	-	3.333±0.29	13.67±0.08	9.67±0.08
5	*Bacillus subtilis*	30.67±0.06	31±0.01	-	-	10333±0.15	23±0.05	14.76±0.04
6	*Bacillus cereus*	33.67±0.11	31.33±0.02	-	-	11.33±0.09	22.67±0.03	13.33±0.16
7	*Escherichia coli*	28.67±0.17	32.33±0.03	-	-	8.67±0.27	26±0.09	19.67±0.15
8	*Klebsiella pneumoniae*	37±0.09	36.67±0.06	-	-	11.333±0.08	31±0.13	19.33±0.06
9	*Salmonella typhimurium*	32.67±0.17	34.33±0.07	-	-	9.33±0.19	19.67±0.22	14.67±0.09
10	*Salmonella typhi*	31±0.07	33.33±0.15	-	-	3.67±0.06	19.33±0.05	11.67±0.03
11	*Pseudomonas aeroginosa*	27.67±0.09	31.33±0.07	-	-	10.333±0.17	22.33±0.08	16.33±0.02
12	*Serratia marcescens*	40±0.14	38.67±0.07	-	-	11.67±0.5	24.33±0.07	16.33±0.04
				Fungal strains				
13	*Candida albicans*	-	-	30.33±0.02	31±0.04	7.32±0.34	21±0.15	13.67±0.14
14	*Candida tropicalis*	-	-	37.33±0.03	33.33±0.13	12±0.08	18.67±0.11	15.33±0.09
15	*Aspergillus fumigatus*	-	-	31.33±0.08	28.67±0.09	7.33±0.03	26.67±0.11	18±0.08
16	*Aspergillus niger*	-	-	34.67±0.12	32±0.05	12.67±0.18	22±0.14	15.67±0.08
17	*Fusarium oxysporum*	-	-	32.33±0.09	29.67±0.23	9.33±0.12	14.33±0.11	11±0.16

Values are mean ± S.E.M. of three replicate experiments (*n*=3); Fractions obtained from hexane, ethylacetate and methanol, respectively.

The experiment was performed under strict asceptic conditions. Inhibition of microbial growth was determined by measuring the diameter of the zone of inhibition. The experiment was performed in triplicates and the mean values of the result are shown in Table 1. Amongst the bacterial strains used Klebisiella pneumonia, E.coli, S.marcescens were found to be most sensitive to Fr-Et than Fr-Me and Fr-He.

The minimum inhibitory concentration of the fractions, studied for microorganisms were enumerated in Table 2. Highest inhibitory of Fr-Et was found against *E. coli*, *P. aeroginosa* and *K. pneumoniae* (MIC values were found 10µg/disc). Inhibitory concentration was higher for *S.epidermis, M. luteus, S.typhimurium, S. marcescens* (MIC values were found 50µg/disc)and Inhibitory concentration was moderate for *S. aureus, S. pyogenes, B.subtilis, B. cereus, S. typhimurium and S. typhi* (MIC values were found 25µg/disc). Among the fungal strains, zone of inhibition *A.fumigatus ans A. niger* were lowest (MIC values were found 25µg/disc).

Table 2. The MIC values (µg/disc) of *C.cretica* fractions against the microorganisms.

Sl. No.		Microorganisms	Hexane fraction	Ethylacetate fraction
Bacterial strains				
1	*Staphylococcus aureus*	250	25	150
2	*Staphylococcus pyogenes*	200	25	100
3	*Staphylococcus epidermidis*	250	50	100
4	*Micrococcus luteus*	250	50	150
5	*Bacillus subtilis*	200	25	100
6	*Bacillus cereus*	200	25	200
7	*Escherichia coli*	200	10	100
8	*Klebsiella pneumoniae*	200	10	100
9	*Salmonella typhimurium*	150	50	100
10	*Salmonella typhi*	200	25	100
11	*Pseudomonas aeroginosa*	150	10	50
12	*Serratia marcescens*	200	50	100
Fungal strains				
13	*Candida albicans*	250	50	150
14	*Candida tropicalis*	200	50	100
15	*Aspergillus fumigatus*	200	25	100
16	*Aspergillus niger*	100	25	100
17	*Fusarium oxysporum*	100	50	100

3.2. *Morphological and biochemical alterations*

The morphological and biochemical alterations induced by the ethylacetate fraction were presented in Table 3. So the further studies of morphological alterations, where the motile organisms like viz., *S.aureus, S.typhi, S. typhimurium* have showed less motility. In the present investigation the inhibitory concentration against $1x10^9$cells has induced the morphological alterations such as the highly motile *S.typhimurium*, *S. typhi* and *S.aureus,* and *E.coli* at 29°C have become sluggish, and some non motile as if they were dead. The

gram staining showed shrivelling of the cells and degeneration. The colony morphology of *E.coli* appeared less viscous after treatment with *C.cretica* indicating reduction or change in the capsular polysacharide material which is an anti phagocytic agent. The smooth colonies of *S. typhi* and *S. typhimurium* have changed to somewhat rough colonies after the drug treatment indicating changes in O-polysaccharide chain of LPS of these pathogens. The drug treated colonies of all pathogens showed reduction in colony size.

Table 3. The morphological and biochemical alterations induced by ethylacetate fraction of *C.cretica.*

Tests	*S. aurious*		*S. typhi*		*S.typhimurium*		*E.coli*	
	Ctrl	C.C. (25μg/ml)	Ctrl	C.C. (25μg/ml)	Ctrl	C.C. (25μg/ml)	Ctrl	C.C. (25μg/ml)
Gram staining	+	+	-	-	-	-	-	-
Motility	+	-	+	-	+	Sluggish/-	+	-
Indole	-	-	-	-	+	+	-	-
Methyl red	+	-	+	-	+	-	+	-
Mac Conkey Agar	-	-	-	-	-	-	-	-
Dnase	-	-	-	-	-	-	-	-
Coagulase	-	-	-	-	+	-	-	-
Mannitol Salt agar	-	-	-	-	+	+	-	-
Catalase	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	+	-	-
Urease	-	-	-	-	+	-	-	-
Amylase	-	-	-	-	+	+	-	-
Protease	-	-	-	-	+	-	-	-
Lipase	-	-	-	-	-	+	-	-
Sucrose	A+,G-	A-,G-	A+,G-	A+,G+	A+,G-	A+,G-	A-,G+	A-,G-
Glucose	A+,G+	A-,G-	A+,G+	A-,G-	A+,G+	A+,G-	A+,G+	A-,G-
Mannitol	A+,G+	A-,G-	A+,G+	A-,G-	A+,G-	A-,G-	A+,G+	A-,G-

A = acid; G = gas; + = positive; - = negative; control = no fraction.

The biochemical alterations induced by *C.cretica* Fr-Et was inhibition of acid formation with various sugars indicating that our fraction has inactivated the enzymes that catalyze these reactions and thereby blocking different fermentation pathways. Similarly the oxidase and urease reactions were negative after treatment. It indicates that the enzymes oxidase and urease were inactivated by ethylacetate fraction. Similarly alterations in methyl red reaction indicates inactivation of enzymes that catalyze mixed acid fermentation reactions, were inactivated there by blocking these pathways. The dnase and phospholipase-C activities of pathogens were also inhibited by treatment indicating the inactivation of this enzyme by *C.cretica*. These results clearly indicate that Fr-Et was a

potent antimicrobial agent that inactivated a large number of enzymes which are the major virulence factors of these pathogens.

4. Conclusion

The fractions of *Cressa cretica* Linn in this study showed a broad spectrum of activity against both gram-positive and gram-negative bacteria and fungi. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent anti-microbial drugs of natural origin. Isolation, identification and purification of these phyto-constituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents are our future direction for investigation.

Acknowledgement

The authors are indebted to the Department of Health and Family Welfare, Government of Jharkhand for its continuous encouragement towards this piece investigation.

References

1. G. G. F. Nascimento, J. Locatelli, P. C. Freitas and, G. L. Silva, Braz. J. Microbiol. **31**, 247 (2000).
2. J. E. Bandow, H. Brotz and, L.I. Leichert, Chemother. **47,** 948 (2003).
3. H. R. El-Seedi, T. Ohara, N. Sata and, S.Nishiyama, J. Ethnopharmacol. **81**, 293 (2002). http://dx.doi.org/10.1016/S0378-8741(02)00101-0
4. A. Braca, M. Politi, R. Sanogo, H. Sanou, I. Morelli, C. Pizza, and, N.D. Tommasi, J. Agric. Food Chem. **51**, 6689 (2003). http://dx.doi.org/10.1021/jf030374m
5. H. J. D. Dorman and S. G. Deans, Flavour. Fragrance J. **15**, 12 (2000). http://dx.doi.org/10.1002/(SICI)1099-1026(200001/02)15:1<12::AID-FFJ858>3.0.CO;2-V
6. H. O. Saxena and M. Brahman, The Flora of Orissa (Capital Business services and consultancy, Bhubaneswar, India, 1995).
7. N. D. Prajapati, S. S. Purohit, A. K. Sharma and, T. Kumar, A handbook of medicinal plants, a complete source book (Agrobios Ltd., India, 2004).
8. P. K. Warrier, V. P. K. Nambier and, C. Ramankutty, Indian Medicinal Plants a Compendium of 500 Species (Council for Scientific and Indusrial Research, New Delhi, India, 1990).
9. A. M. Rizk and H. I. Heiba, Int. J. Crude Drug Res. **28**, 89 (1990).
10. A. M. Rizk and G. A. El-Ghazaly, Medicinal and Poisonous Plants of Qatar (University of Qatar, Scientific and Applied Research Centre, Qatar, UAE, 1995).
11. A. A. Shahat, N. S. Pieters and, L. A. J. Vlietinck, Pharma. Biol. **4**, 349 (2004). http://dx.doi.org/10.1080/13880200490519622
12. P. Sunita, S. Jha and, S. P. Pattanayak, Middle-East J. Sci. Res. **8**, 129 (2011).
13. P. Sunita, S. Jha and, S. P.Pattanayak, Der Pharma. Lettre **3**, 259 (2011).
14. P. Sunita, S. Jha and, S. P.Pattanayak, Pharm. Res. **1**, 157 (2009).
15. S. Priyashree, S. Jha and, S. P. Pattanayak, Pharma. Rev. **4**, 161 (2010). http://dx.doi.org/10.4103/0973-7847.70910
16. W.M. Kirby, A. W. Bauer, J. C. Sherries and, M. Turck, Am. J Clin. Pathol. **45**, 493 (1996).
17. J. G. Cappuccino and N. Sherman, Microbiology a Laboratory Manual (Addison Wesley Longmann Inc., 1999).
18. D. S. Ogunleye and S. F. Ibitoye, Tropical J. Pharm. Res. **2**, 239 (2003).

19. H. Finnermore, J. M. Cooper, M. B. Stanley, J. H. Cobcroft and, L. J. Harris, J. Soc. Chem. Ind. **57**, 162 (1988).
20. M. R. Berenbaum, J. K. Nitao and, A. R. Zangerl, J. Chem. Ecol. **17**, 207 (1991). http://dx.doi.org/10.1007/BF00994434
21. I. Weinmann. History of the development and applications of coumarin and coumarin-related compounds (O'Kennedy & Thornes, UK, 1997).
22. S. Sardari, Y. Mori, K. Horita, R. G. Micetich, S. Nishibe and, M. Daneshtalab, Bioorg. Med. Chem. **7,** 1933 (1999). http://dx.doi.org/10.1016/S0968-0896(99)00138-8
23. U. Matern, P. Luer and, D. Kreusch, Biosynthesis of coumarins,In: D. Barton, K. Nakanishi, O. Meth-Cohn and, Sankawa (Elsevier Science Ltd., Oxford, UK 1990).
24. S. Nurdan and U. aysel, Eurasian J. Biosc. **4**, 28 (2007).
25. J. K. Todd, Clin. Microbial. Rev. **1**, 432 (1998).
26. R. Hajjeh, A. Reingold and, A. Weil, Emer. Infect. Diseases **5**, 807 (1999). http://dx.doi.org/10.3201/eid0506.990611
27. R. J. Rubin, C. Harrington, A. Poon, K. Dietrich, J. Grene and, A. Moiduddin, Emer. Infect. Diseases **5,** 9 (1999). http://dx.doi.org/10.3201/eid0501.990102
28. R. Hema, S. Kumaravel and, N. Elanchezhiyan, Global J. Pharmacol. **3**, 38 (2009).
29. H. K. Kaufman, Geriatrics **52**, 50 (1997).
30. S. Friedman, S. E. Richardson, S. E. Jacobs and, K. O'Brien, Ped. Infect. Disease J, **19**, 499 (2000). http://dx.doi.org/10.1097/00006454-200006000-00002
31. G. L. Young and D. Jewell, Cochrane Database System Rev. **2**, 225 (2000).
32. J. W. Rippon, Candidiasis and the pathogenic yeasts, In: J. W. Rippon ed. (Med Mycol, PA, 1988).
33. W. A. Kennedy, C. Laurier, D. Gautrin, H. Ghezzo and, M. J. L. Pare, J. Clin. Epidem. **53**, 696 (2000). http://dx.doi.org/10.1016/S0895-4356(99)00191-2